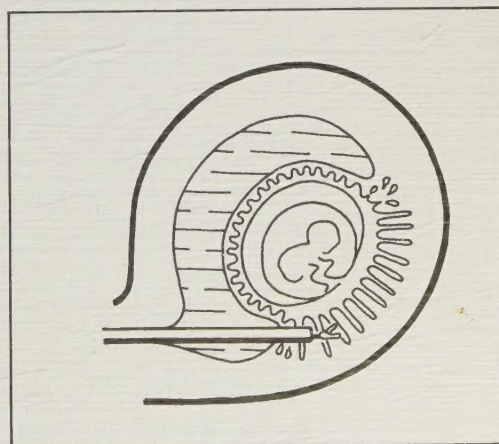
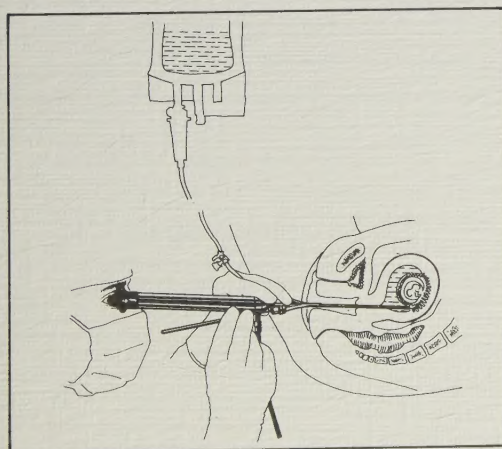
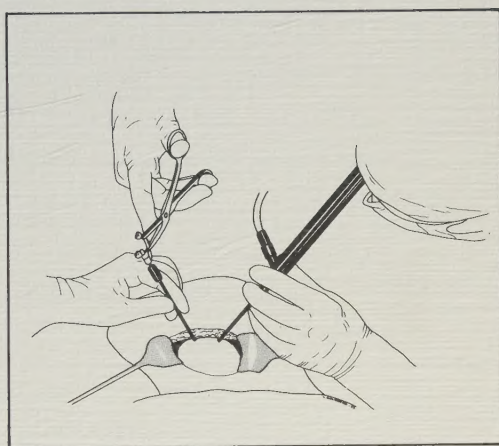
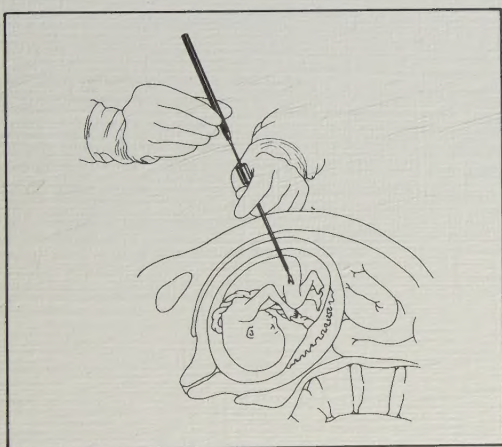


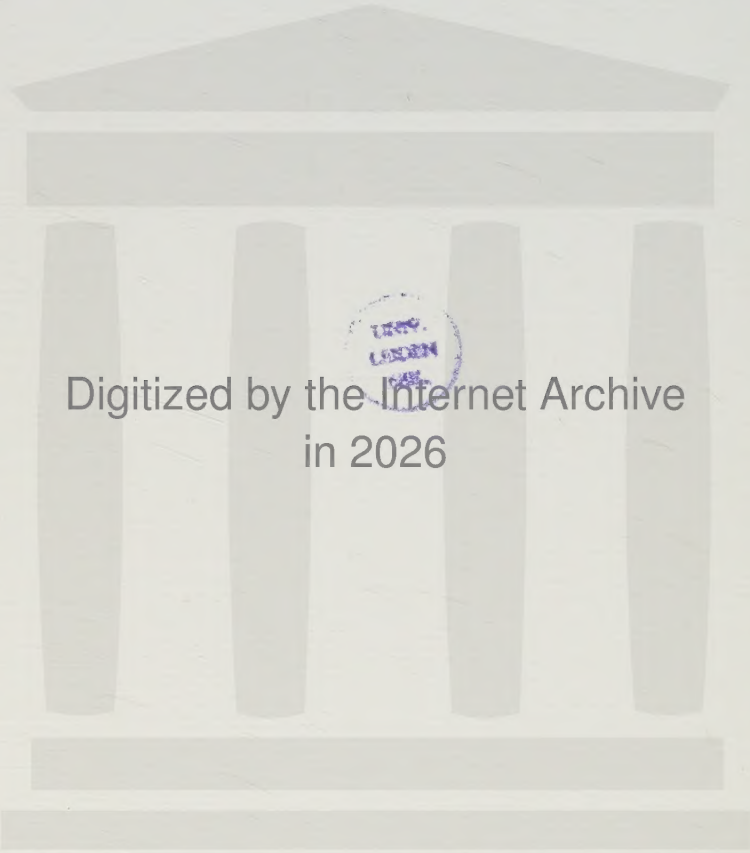
Techniques for Sampling Fetal Skin, Fetal Muscle or Chorionic Villi for Prenatal Diagnosis

by

Lars Löfberg



Lund 1984



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TECHNIQUES FOR SAMPLING FETAL SKIN,
FETAL MUSCLE OR CHORIONIC VILLI
FOR PRENATAL DIAGNOSIS

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Cover figures

- (top left) "Blind" sampling of fetal skin.
Drawing: Louise Goldberg.
- (top right) Direct vision sampling of either skin or
muscle. Drawing: Louise Goldberg.
- (bottom) Chorionic villi sampling.
Drawings: Ingemar Nilsson.

The studies were supported by grants from the Medical
Faculty of Lund University

Printed in Sweden
Studentlitteratur
Lund 1984

Dedicated to my wife Eva, and to Gustaf and Erik

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ABSTRACT

The purpose of the study was to develop reliable methods for clinical in utero sampling of fetal skin, fetal muscle or chorionic villi.

Fetal skin biopsy. "Blind" biopsy procedure, the conventional method for fetal skin sampling in utero, involves the trans-abdominal insertion of a fetoscope into the amniotic sac, selection of a site suitable for skin biopsy, replacing of the fetoscope with a biopsy forceps, and, finally, "blind" sampling. This method involves a risk of inadvertently collecting fetal membranes (amnion, chorion) or placental and uterine wall fragments instead of skin. A technique was developed that enables fetal skin biopsy to be performed with direct vision by means of two cannulas, one for the optic instrument and the other for the biopsy forceps. The two methods were compared in cases scheduled for second-trimester elective abortion by hysterotomy. Of 45 specimens obtained "blind", only nine (1:5) consisted of skin, whereas all 17 specimens taken under direct vision were of skin. The direct vision technique was successfully applied on 4 fetuses at risk for severe skin disorders. The presence of the disorders could be excluded and, after uneventful pregnancies, healthy infants were born in due course.

Fetal muscle biopsy. No reliable in utero test has been available for inherited muscular dystrophies, of which Duchenne muscular dystrophy is the commonest and most severe. However, examination of fetal muscle obtained ex utero from male fetuses at risk, and therapeutically aborted in the second trimester, has revealed histological and histochemical alterations compatible with Duchenne muscular dystrophy. A technique was developed for fetal muscle sampling that involves the removal, under direct vision, of a small piece of skin, thereby exposing the underlying muscle. The technique was studied in cases scheduled for second-trimester elective abortion by hysterotomy. Of the 14 specimens collected, all contained



muscle fibers. Each specimen measured about 0.5 to 1.0 mm across and contained 300 to 900 fibers. The material was sufficiently large and well-preserved to allow application of those stainings and enzyme reactions routinely used in the postnatal diagnosis of Duchenne muscular dystrophy.

Chorionic villi sampling. The technique most commonly used for sampling of chorionic villi in the first trimester is ultrasound-guided trans-cervical aspiration. One disadvantage with this method is that the specimen obtained may be contaminated with maternal tissue, a possible source of error in diagnosis. A technique was developed for sampling chorionic villi under direct vision. The technique involves the distension of the extra-amniotic space with physiologic saline solution, thus giving a clear view of the amniotic sac and the intended biopsy site. The technique was studied in women scheduled to undergo first trimester elective abortion by vacuum aspiration. Contamination with maternal tissue was found to be easily avoided, and the increase in intra-uterine pressure caused by the distension of the extra-amniotic space was found to be much the same as that of the ordinarily painless, physiologic Braxton Hicks contractions. The technique was used for diagnosis in 41 cases, in all of which the samples obtained were free from contamination with maternal tissue. To date (February, 1984), of the 36 women who continued their pregnancy after sampling, 2 women have aborted spontaneously, one three weeks and the other five weeks after the sampling, 4 have given birth to healthy infants, and the remaining 30 women have continued their pregnancy uneventfully.

This thesis is based on the following papers:

- I Löfberg L, Gustavii B
Technical difficulties in fetal skin sampling.
Acta Obstet Gynecol Scand 1982; 61:505-7.
- II Löfberg L, Gustavii B
"Blind" versus direct vision technique for fetal
skin sampling in cases for prenatal diagnosis.
Clin Genet 1984; 25:37-41.
- III Gustavii B, Löfberg L, Henriksson K G
Fetal muscle biopsy.
Acta Obstet Gynecol Scan 1983; 62:369-71.
- IV Gustavii B, Chester M A, Edvall H, Iosif S,
Kristoffersson U, Löfberg L, Mineur A, Mitelman F
First-trimester diagnosis on chorionic villi
obtained by direct vision technique.
Hum Genet 1984; 65:373-6.
- V Löfberg L, Iosif C S, Edvall H, Gustavii B
Direct vision sampling of chorionic villi during
extra-amniotic instillation of physiologic saline
solution. Effect on intra-uterine pressure and
fetal heart activity. Submitted for publication.

INTRODUCTION

The purpose of the present study was to develop clinically useful methods for in utero sampling of fetal skin, fetal muscle or chorionic villi. The study was carried out in cases for elective abortion, and, when practicable, the methods were applied in cases for diagnosis.

Fetal skin biopsy

In 1980 Elias and co-workers reported the first successful fetal skin sampling in utero in a case requiring prenatal diagnosis. Elias took the skin specimen "blind", a technique that has since become the conventional method for fetal skin sampling (Anton-Lamprecht et al., 1981; Esterly & Elias, 1983; Golbus et al., 1980; Löfberg et al., 1980, 1983). One disadvantage of the procedure is that tissue specimens may be inadvertently removed from fetal membranes, the myometrium, or even the trophoblast. Elias, in his first attempt, obtained solely a piece of the amniotic membrane and thus no skin specimen; (4 weeks later he repeated the fetoscopy successfully). A technique was developed at our department enabling biopsy of the skin to be effected under direct vision by means of two cannulas, one for the optic instrument and the other for the biopsy forceps. In the present study of skin sampling in utero, we tested the two methods in cases for second-trimester elective abortion by hysterotomy. The direct vision technique was then applied in cases for diagnosis.

Fetal muscle biopsy

No reliable in utero test for inherited muscular dystrophies is available. In the commonest and most severe of these disorders, Duchenne muscular dystrophy, measurement of creatine phosphokinase activity in fetal serum obtained at fetoscopy in the second trimester of high-risk pregnancies has been used as a diagnostic test. However,

Emery (1979) and Golbus (1979) and their co-workers found that the disease was not manifested in all affected cases by elevated creatine phosphokinase activity in the second trimester. It is therefore not recommended that Duchenne muscular dystrophy be diagnosed solely on the basis of fetal serum creatine phosphokinase activity.

On the other hand, examination of fetal muscle obtained ex utero from male fetuses at risk, and therapeutically aborted in the second trimester, has revealed histological and histochemical alterations compatible with Duchenne muscular dystrophy (Brambati et al., 1980; Emery, 1977; Emery et al., 1979, 1980; Toop & Emery, 1974; Winn & Heller, 1978).

A technique was developed at our department enabling biopsy specimens of fetal muscle to be obtained in utero. Experience of the method was obtained in second trimester hysterotomy abortions.

Chorionic villi sampling

Simoni et al. (1983) tested various approaches for the sampling of chorionic villi. They concluded that the technique of blind catheter aspiration presented by Old et al. (1982) might be the best method. With the blind technique, however, up to four aspiration attempts may have to be made to obtain adequate material (Old et al., 1982; Pergament et al., 1983), and in the study by Simoni et al. (1983) from 30 min up to 1 h was needed to separate villi from maternal decidual fragments. Moreover, attempts at separation may not always be successful or the results reliable (Tietung, 1975; Niazi et al., 1981).

A technique was developed at our department for the sampling of chorionic villi under direct vision. The technique involves instilling physiologic saline solution into the extra-amniotic space, thus giving a clear view of the amniotic sac and the intended biopsy site. The technique was studied in women scheduled to undergo first-trimester elective abortion by vacuum aspiration. The method was then applied in cases requiring diagnosis.

MATERIAL AND METHODS

Fetal skin biopsy

Methodology study

In 14 women scheduled to undergo elective abortion by hysterotomy in the 16th to 21st week of gestation, removal of skin biopsy samples from the fetus was attempted. In the first 10 women the biopsy was taken "blind", and in the remaining 4 women it was obtained under direct vision.

Diagnostic cases

The clinical material included 7 cases of skin sampling from the fetuses of 6 women (Table I). In one of the women, the examination was done in two consecutive pregnancies (cases 2 and 4 in Table I). In the first 3 cases, the skin biopsies were taken "blind"; in the remaining 4 they were obtained by a two-cannula technique that permits sampling of the skin under direct vision.

Case 1: The woman had one healthy boy and a girl who suffered from the Herlitz syndrome (epidermolysis bullosa atrophicans generalisata gravis = epidermolysis bullosa letalis) and who died from sepsis at the age of 19 days. Both parents must be regarded as heterozygous carriers of the Herlitz gene, which is transmitted in an autosomal recessive pattern. The parents were informed of the 1:4 risk of their having a child affected by the disease. In a subsequent pregnancy the woman requested prenatal diagnosis. In the 19th week, fetal skin specimens were obtained at fetoscopy.

Cases 2 and 4: The woman herself suffers from bullous congenital ichthyosiform erythroderma (bullous ECI). From the first day of her life, serous blisters and patchy superficial epidermolysis had appeared all over her body. New bullae appeared almost daily in her first decade. Due to secondary infections, she had been frequently hospitalized, especially in childhood. As an adult, she had blister eruptions, with free intervals of only a few weeks. During

her first year of marriage, the woman avoided becoming pregnant because of her fear of having an affected child, the risk of which is 1:2. Then, after having received genetic counselling, she and her husband considered pregnancy, but only because of the availability of prenatal diagnosis of the disorder. In two consecutive pregnancies, fetal skin specimens were obtained at fetoscopy, which, in both cases, was carried out in the 20th week of pregnancy.

Cases 3, 5 and 6: These cases resemble case 1, with one baby of each woman suffering from Herlitz syndrome and succumbing at the age of 2 to 5 months. In each of these cases, in the 19th week of a subsequent pregnancy, fetal skin specimens were taken at fetoscopy.

Case 7: The couple had a son with non-bullous congenital ichthyosiform erythroderma (non-bullous ECI). They felt that they should not risk having a second affected child, and the woman had avoided becoming pregnant. However, after having been informed of the possibility of prenatal diagnosis the couple considered another pregnancy. In the 21st week of that pregnancy, fetal skin specimens were taken at fetoscopy.

Technique

The "blind" biopsy technique: A sharp trocar and cannula (diameter 2.2 mm) were introduced percutaneously into the amniotic cavity under general or local anesthesia. The trocar was withdrawn and a "Needlescope" (Dyonics, Inc.), 1.7 mm in diameter, was inserted through the cannula. A site suitable for skin biopsy was chosen. The cannula was placed gently on the fetal skin at the selected site. The fetoscope was withdrawn, a biopsy forceps (2x2 mm jaws) was then passed down the cannula and biopsy specimens were obtained "blind" (cover page, top left figure).

The direct vision technique: Under general anesthesia the uterus was exposed by a low abdominal incision. A sharp trocar and cannula were introduced through the myometrium into the amniotic cavity. The trocar was removed and a Needlescope was inserted through the cannula. A site

suitable for biopsy of the skin was selected. A second trocar and cannula were then introduced into the amniotic cavity, 3-5 cm from the site of insertion of the first Needlescope. The trocar was withdrawn and a second Needlescope was passed down and guided visually towards the selected site. The second Needlescope was then replaced with a biopsy forceps, and biopsy specimens were taken under direct vision (cover page, top right figure).

Microscopy.

The specimens were immediately placed in fixative solution. Those from the first 5 cases for elective abortion and from the 7 diagnostic cases were sent to Heidelberg and there processed and examined under the electron microscope by Professor Ingrun Anton-Lamprecht (Anton-Lamprecht, 1981, 1983). The samples from the remaining cases were processed in Lund; they were embedded in paraffin wax, sectioned, stained with eosin-hematoxylin and by van Gieson's method, and then examined in a light microscope.

Fetal muscle biopsy

The fetuses of 7 women scheduled to undergo elective abortion by hysterotomy in the 16th to 20th week of gestation were studied.

Technique

Under general anesthesia, the uterus was exposed by a low abdominal incision. A sharp trocar and cannula (diameter 2.2 mm) were introduced through the myometrium into the amniotic cavity. The trocar was removed and a 1.7 mm diameter Needlescope was inserted through the cannula. A site suitable for muscle biopsy was chosen (thigh or calf). A second trocar and cannula were then introduced into the amniotic cavity about 3-5 cm from the insertion site of the first Needlescope. The trocar was withdrawn and a second Needlescope was passed down and guided by direct vision towards the selected site.

The second Needlescope was then replaced by a biopsy forceps (2x2 mm jaws). Under direct vision, a small piece of skin was removed and two biopsy specimens were obtained from the underlying exposed muscle (cover page, top right figure).

Examination of specimens

The specimens were immediately dipped in talcum, frozen in liquid nitrogen, and stored at -70°C until analysed.

The following stains and enzyme reactions were employed: hematoxylin-eosin, van Gieson, myofibrillar ATPase at pH 9.4 and 4.6, and alizarin red S. Fiber diameter and fiber area were determined by a digitizer connected to a calculator (Sandstedt, 1981).

Chorionic villi sampling

Methodology study

The methodology study included 56 women scheduled to undergo first-trimester elective abortion by vacuum aspiration. In 48 of them, chorionic villi were sampled, and, during sampling, fetal heart activity was registered; in the remaining 8 controls, intra-uterine pressure was recorded during instillation of saline into the extra-amniotic space.

Diagnostic cases

In 41 women in the first trimester, chorionic villi were sampled for the purpose of karyotyping or analysis of enzyme activity. In the first 5 women, fetal heart activity was checked, but not counted, during sampling; in the remaining 36 women, fetal heart activity was counted.

Registration of fetal heart activity

Before, during and a few minutes after sampling, fetal heart activity per 15 seconds was counted on real-time ultrasound scans made with a Hitachi EUB-25M Linear Array or Diasonics DS 20 Sector Scanner.

Intra-uterine pressure recording

A double catheter system was used, i.e. a No. 12 Foley catheter within which a 2 mm diameter micro-transducer catheter (Gaeltec) had been placed. In the external orifice of the Foley catheter a specially manufactured occlusive plastic cuff was inserted, encircling the micro-transducer catheter.

The catheters were introduced, with the Foley catheter balloon lying just inside the internal cervical orifice. The balloon was distended with 5 ml of fluid. During continuous recording of the intra-uterine pressure with a Watanabe Linear Corder, Mark III, an amount of 100-150 ml of saline was instilled extra-amniotically through the Foley catheter by a volumetric infusion pump (Imed, Milton Trading Estate) at a rate of 10 ml per minute.

The micro-transducer catheter was calibrated before and after the recording.

The reason for using up to 150 ml of saline in this study was our previous finding during sampling in women scheduled to undergo vacuum aspiration that the amount of saline needed to distend the extra-amniotic space, as calculated from ultrasound scans, was 150 ml at most.

Technique of sampling chorionic villi

The equipment used was a 1.7 mm diameter endoscope (Olympus Selfoscope or Dyonics Needlescope) and a specially manufactured endoscope cannula 3.0 x 4.7 mm in outer diameter (Richards Scandinavia, Mölndal, Sweden) with two attachments, one for the instillation of body-temperature saline solution and the other for the biopsy forceps (pencil-type grip, Olympus or Dyonics), and a rubber hood covering the forceps attachment.

With the endoscope positioned in the cannula and the saline "drip" functioning, the set of instruments was introduced into the extra-amniotic space and directed to the base of the amniotic sac. The biopsy forceps were inserted and a piece of decidua removed (cover page, bottom left figure). The forceps were withdrawn and, under endoscopic

vision, the instrument was inserted 1 to 2 cm into the placenta. A second pair of forceps was passed through, with the jaws just emerging from the internal orifice of the cannula (the second pair was used to avoid any contamination with maternal tissue adhering to the jaws of the forceps first used). An assistant made sure with ultrasound that the tip of the instrument was located half-way between the base of the amniotic sac and the base of the placenta (cover page, bottom right figure). The forceps were rotated once or twice, thereby entwining villi around the jaws. The jaws were then closed and, to avoid any stripping of the specimen, the forceps were held in position by an assistant while the saline "drip" was stopped, the endoscope withdrawn and the rubber hood loosened from its attachment. Finally, the forceps were carefully withdrawn.

In the first 7 diagnostic cases studied, the sampling procedure was done under general anesthesia; thereafter, in 6 under local anesthesia; in 14 after 15 mg Diazepam by rectal instillation 20 minutes before the procedure and 10 mg Diazepam intravenously on commencing; and, finally, in 14 with neither anesthesia nor analgesia.

RESULTS AND COMMENTS

Fetal skin biopsy

In the methodology study, of 45 biopsy specimens obtained "blind", 9 specimens (thus only one out of every five) consisted of skin. Of the remaining 36 specimens, 26 consisted of fetal membranes; 6, of myometrium; and 4, of trophoblast. By contrast, all 17 specimens taken under direct vision were of skin.

The results of the diagnostic cases are presented in Table I. On the basis of the ultrastructural examination, one of the fetuses at risk of having bullous ichthyosiform erythroderma (case 2 in Table I) was found to be affected. In the remaining 6 fetuses at risk, the presence of the disorders was excluded.

The pregnancy of the affected fetus was terminated by hysterotomy (Dr. Kåre Molne, Rikshospitalet, Oslo). The 480 g fetus had skin lesions on wrist and hyperkeratosis on scalp, face, palms, and soles. Barely visible punctate changes in the skin on the right hip and the right buttock suggested the sites of the fetoscopy biopsies carried out 16 days previously. Light and electron microscopy of skin specimens from the abortus confirmed the diagnosis of bullous ichthyosiform erythroderma.

Five of the six pregnancies with unaffected fetuses proceeded uneventfully to parturition at term. In the remaining case (case 3 in Table I), where the "blind" technique was used, the woman was hospitalized from the 26th week of gestation because of intermittent leakage of amniotic fluid. Labor started in the 33rd week, and a healthy boy weighing 2.02 kg was delivered by cesarean section. In this case, only 2 of the 8 biopsies taken were of skin; the remaining 6 were fetal membranes in 3 of the cases, myometrium in 2, and trophoblastic tissue in 1.

The 6 children are developing normally, the oldest now being 3 years old. Barely visible scars, suggesting the

Table 1. Diagnostic cases of fetal skin sampling

Case No. *	Gestational age (weeks)	Genetic risk	No. of biopsies	Tissue obtained				Fetal diagnosis	Outcome
				Skin	Fetal membranes†	Myometrium‡	Trophoblast‡		
<i>"Blind" biopsy</i>									
1	19	Herlitz syndrome§	8	6	2	0	0	Normal	Term delivery -- normal newborn skin
2	20	Bullous ECI§	10	7	0	1	2	Bullous ECI	Bullous ECI in abortus
3	19	Herlitz syndrome	8	2	3	2	1	Normal	Premature delivery (33rd week) -- normal newborn skin
<i>Biopsy under direct vision</i>									
4	20	Bullous ECI	3	3	0	0	0	Normal	Term delivery -- normal newborn skin
5	19	Herlitz syndrome	2	2	0	0	0	Normal	Term delivery -- normal newborn skin
6	19	Herlitz syndrome	2	2	0	0	0	Normal	Term delivery -- normal newborn skin
7	21	Non-bullous ECI	2	2	0	0	0	Normal	Term delivery -- normal newborn skin

* Cases numbered in order in which they were studied.

† This specimen consisted of either amnion alone or both amnion and chorion.

‡ In most cases this specimen included fetal membranes.

§ Herlitz syndrome = epidermolysis bullosa atrophicans generalisata gravis = epidermolysis bullosa tetalis.

§ ECI = congenital ichthyosiform erythroderma.

sites of biopsy, were found in 2 of the newborns (cases 5 and 7 in Table I).

The time needed for processing of the biopsies and for diagnostic electron microscopy was, in the cases at risk of Herlitz syndrome, 7-10 days; in the cases at risk of ichthyosiform erythroderma, 11-13 days.

The "blind" biopsy procedure has several serious drawbacks. First, specimens of tissues other than skin may be inadvertently removed if the tip of the cannula slips off the fetus and onto the uterine wall or the placenta. Injury resulting from such unsuccessful biopsies can cause miscarriage or premature birth. Second, several biopsy specimens may have to be taken to ensure that enough skin material will be available for the microscopic examination. Third, due to the risk of injury to the face (eyes, lips, nose, etc.), the "blind" method is unsuitable for biopsy attempts from the scalp, the site where the first ultrastructural signs of keratinization disturbance ought to appear, according to the work of Holbrook (1979) and Holbrook & Odland (1980) on normal fetal skin development.

However, the two-cannula technique (one cannula for the optic instrument and the other for the biopsy forceps) permits biopsy of the skin under direct vision. With this method, used in the last four fetoscopies in the present study, we were able not only to take the biopsy specimens from the scalp in 2 cases at risk of ichthyosiform erythroderma but also to confine the number of biopsies to two or three.

To the best of our knowledge, the only previous report on fetal skin sampling under direct vision is that of Rodeck (Rodeck et al., 1980). He used a specially constructed biopsy forceps which is so tiny that it can be passed through the cannula together with the fetoscope. But this tiny forceps is not commercially available. However, the instruments used in the two-cannula procedure presented in this report are all available commercially.

Fetal muscle biopsy

Examination by microscopy of the 14 biopsy specimens from the 7 fetuses revealed that all contained muscle fibers. Each measured about 0.5 to 1.0 mm across and contained 300 to 900 fibers. Mean fiber diameter was $9.9 \pm 1.1 \mu\text{m}$ (range 8.6-12.1) and mean fiber area $110 \pm 18 \mu\text{m}^2$ (range 90-140). The material was sufficiently large and well-preserved to allow application of those stainings and enzyme reactions attempted (see Methods). No eosinophilic or hyaline fibers and no calcium-positive fibers were identified. Post-abortem examination of the biopsy site showed that the diameter of the skin lesions was about 2 mm; the muscle lesions were barely visible.

With this technique it has proved possible to obtain biopsies of fetal muscle. The specimens were taken from large muscles, such as thigh or calf. Wounds from surgery in utero heal rapidly (Hodgen, 1981). Skin biopsies performed on the fetus in the second trimester appear not to leave any prominent scars or sequelae visible after birth. It has also been shown that muscle tissue has a great capacity to regenerate (Allbrook, 1981).

With the exception of the work by Brambati et al. (1980), previous investigations of muscle from normal fetuses have included some necropsies from prostaglandin or saline abortuses. It is questionable if values obtained from such specimens can be used as a reference. In the work by Brambati et al., whole (tendon-to-tendon) muscles of hysterotomy abortuses were removed and immediately frozen. In such preparations, reliable values of fiber diameter and variation in fiber diameter can be obtained. Nevertheless, these data are not suitable for comparison with those from small biopsies, such as fetoscopy samples, since whole-muscle preparations are easier to handle (so avoiding contractions) than are small biopsy specimens (contractions affect both the diameter of the fibers and their stainability). In the present study, the mean fiber diameter ($9.9 \pm 1.1 \mu\text{m}$) obtained from fetoscopy samples was found to be

larger than that obtained from muscles of post-abortem fetuses (Winn & Heller, 1978; Mahoney et al., 1977; Emery et al., 1979, 1980; Brambati et al., 1980).

In the second-trimester fetuses affected by Duchenne muscular dystrophy, muscle pathology is not characterized by muscle fiber necrosis, muscle fiber regeneration, or an increased amount of fat or connective tissue, as is the case in affected boys (Emery, 1977, Winn & Heller, 1978). In fetuses, the diagnosis has to be based on indices such as increased fiber size, increased variation in fiber size, and increased proportions of eosinophilic, hyaline, and calcium-positive fibers (Brambati et al., 1980; Emery, 1977; Emery et al., 1979, 1980; Toop & Emery, 1974; Winn & Heller, 1978).

In two at-risk fetuses presumed to be affected, Emery & Burt (1980) found that the content of eosinophilic fibers was 6.9-8.0% and that of calcium-positive fibers (alizarin stain) 8.4-12.0%. In a similar study of two other such fetuses, Brambati and co-workers (1980) found 3.5-4.3% hyaline fibers and 3.8-4.8% fibers containing calcium. Histopathological changes occurring in that range ought to be detectable in biopsy specimens of the size obtained in the present study.

We have thus presented a technique suitable for fetal muscle biopsy. At present, this method is not recommended for clinical use. Before such use, the reference values for fetoscopy samples obtained from normal fetuses in the present study have to be compared with those of fetoscopy samples obtained from at-risk fetuses of women who have decided to terminate their pregnancy.

Comments on open abdomen fetoscopy

The sampling of fetal skin by the direct vision technique and the sampling of fetal muscle was carried out under general anesthesia with the uterus exposed by a low abdominal incision. Open abdomen fetoscopy has several advantages. First, the fetoscope can be inserted leaving a

good margin to the large myometrial vessels, damage to which can cause bleeding into the amniotic cavity, thereby impairing vision and making sampling difficult or impossible. Second, penetration of the leathery amniotic membrane is facilitated by the ability to insert the fetoscope exactly perpendicular to the amniotic sac and to apply the thrust at the right moment. Third, in cases of anterior placenta, the fetoscope can be introduced through the posterior uterine wall, thereby avoiding damage to the placenta. Fourth, insertion of the fetoscope can be done more precisely over the site that, judged by ultrasound, seems to be the most suitable one for the sampling. Fifth, the woman is caused less discomfort when under general anesthesia than when undergoing fetoscopy under local anesthesia. We feel that these advantages outweigh the disadvantages of greater cost and a few days' prolonged hospital care.

Chorionic villi sampling

It was found that contamination with maternal tissue could be easily avoided. In all the 41 diagnostic cases, uncontaminated samples of chorionic villi were obtained. Most extra-placental villi, which are technically simpler to obtain than placental villi, were found to be enlarged and edematous, and they contained few or no vessels and had no buds on their surface. Attempts to cultivate such extra-placental villi (destined to degenerate) usually failed, but they were useful for enzyme determination, and for sexing by X- and Y-chromatin examination. It proved possible to pick out by direct vision such villi likely to be those most capable of growing in vitro, i.e., placental villi obtained 1 to 2 cm from the edge of the placenta. Such villi are thin, rich in blood vessels, and have numerous buds. These villi grew in vitro and analysable metaphase plates were obtained within 2 to 3 weeks.

Intra-uterine pressure increased continuously and smoothly during the extra-amniotic saline instillation in the study of cases for elective abortion (Table II). Even

during distension by 150 ml of saline, intra-uterine pressure never exceeded 23 mmHg, thus not more than that previously recorded during the physiologic and ordinarily painless Braxton Hicks contractions (Caldeyro-Barcia & Poseiro, 1958).

Table II. Intra-uterine pressure (mmHg) during instillation of physiologic saline solution into the extra-amniotic space of 8 women scheduled to undergo vacuum aspiration in the 9th to 13th week of gestation.

Case No.*	Gestational age (weeks)	Volume of saline instilled (ml)			
		0	50	100	150
1	10	8	14	20	nd
2	9	10	10	20	nd
3	11	9	9	16	18
4	13	8	8	15	17
5	9	8	8	13	23
6	9	8	8	12	16
7	9	8	8	16	20
8	9	12	12	16	18
Mean		9	10	16	19

* Cases numbered in order in which they were studied.
nd = not done

The results of the registration of fetal heart activity during sampling in the study of women for elective abortion did not differ significantly from those of the diagnostic cases, presented in Table III. Although, with this sampling technique, the extra-amniotic space was distended with saline and the instrument inserted into the placenta under continuous saline infusion, fetal heart activity decreased only slightly. And when sampling was completed and saline allowed to escape, the activity soon returned to pre-sampling values. It is therefore unlikely that the use of this technique has any detrimental effect on the fetus.

Table III. Fetal heart activity (beats per 15 seconds) during chorionic villi sampling in 36 cases for prenatal diagnosis.

	Fetal heart activity		
	range	mean	SD
Before sampling	30 - 40	36.0	2.1
During sampling	25 - 37	33.2	2.6
A few minutes after sampling	32 - 41	36.8	2.2

The technique was used for diagnosis in 41 cases. Of the 36 women who continued their pregnancy after sampling, 2 aborted spontaneously 3 and 5 weeks, respectively, after the procedure; 4 have given birth to healthy babies; and the remaining 30 pregnancies have up to date (February, 1984) continued uneventfully.

The sampling could be performed without anesthesia or analgesia. It caused little or no discomfort and was completed within a few minutes. No cervical dilatation was necessary - not even in nulliparae. The women could return home shortly afterwards.

GENERAL SUMMARY

The results of the present study may be summarized as follows.

Fetal skin sampling

The "blind" biopsy procedure, the conventional method for obtaining fetal skin specimens in utero, was found to involve several drawbacks:

- the tip of the cannula often slips off the fetus and onto the uterine wall or the placenta, from which the biopsy specimen is then inadvertently removed;
- injury resulting from such unsuccessful biopsies can cause miscarriage or premature birth;
- several biopsy specimens may have to be taken to ensure that enough skin material will be available for the microscopic examination;
- due to the risk of injury to the face (eyes, lips, nose etc.), the "blind" method is unsuitable for biopsy attempts from the scalp, the site where the first ultrastructural signs of keratinization disturbance are thought to appear.

A technique was developed that enables biopsy of the skin to be effected under direct vision. With this technique

- all biopsy specimens obtained were of skin;
- the number of biopsies could be confined to two or three;
- biopsies could be taken from the scalp without risk of injuring the face;
- the technique was successfully applied in cases for prenatal diagnosis.

Fetal muscle biopsy

A technique was developed for obtaining biopsy specimens of fetal muscle in utero. With this technique, the muscle material obtained was sufficiently large and well-preserved to allow application of those histological and histochemical

methods that are routinely used to diagnose Duchenne muscular dystrophy, the commonest and most severe disorder among the inherited muscular dystrophies.

Chorionic villi sampling

A direct vision technique was developed for sampling chorionic villi in the first trimester. The technique involves instilling physiologic saline solution into the extra-amniotic space. With this technique

- contamination with maternal tissue was found to be easily avoided;
- fetal heart activity decreased only slightly during sampling and returned to pre-sampling values soon after the procedure had been completed;
- the increase in intra-uterine pressure by the distension of the extra-amniotic space with saline was found to be in the same range as that of the ordinarily painless, physiologic Braxton Hicks contractions;
- it was possible to pick out by direct vision those chorionic villi likely to be those most capable of growing in vitro, i.e., villi rich in blood vessels and with numerous buds on their surface;
- the technique was successfully applied in cases for prenatal diagnosis.

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TECHNICAL DIFFICULTIES IN FETAL SKIN SAMPLING

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Abstract. Attempts were made to obtain skin biopsies from the fetuses of 18 women in the 16th to 21st week of gestation. In four of them the purpose was prenatal diagnosis because of the risk that the fetus might be affected with a severe skin disorder; the remaining women were to undergo elective abortion by hysterotomy. In the first 13 cases the conventional "blind" biopsy procedure was used. Of 71 biopsy specimens obtained with this technique, only one out of every three consisted of skin; the remainder comprised fetal membranes, myometrium, or trophoblast. In one of the diagnostic cases where the "blind" procedure was used, inadvertent removal of tissue specimens from the amniotic sac was probably responsible for the intermittent leakage of amniotic fluid that occurred in this woman from the 26th week of gestation and for the premature delivery in the 33rd week. In the remaining five women (including one for diagnosis), a two-cannula procedure was employed (one cannula for the optic instrument and the other for the biopsy forceps), permitting biopsy of the skin under direct vision. All the biopsy specimens taken by this method consisted of skin.

The "blind" biopsy procedure is the most common method for obtaining a fetal skin specimen in utero (1–7). A great disadvantage of this procedure is that tissue specimens may be inadvertently removed from the fetal membranes (4), the myometrium, or even the trophoblast (1). This paper describes a technique of using two cannulas, one for the optic instrument and the other for the biopsy forceps, thereby enabling fetal skin sampling to be performed under direct vision.

CLINICAL MATERIAL AND METHODS

In 18 women in their 16th to 21st week of gestation (menstrual weeks), removal of skin biopsy samples from the fetus was attempted. In four of them the purpose of the biopsy was diagnostic, i.e., to demonstrate or exclude the presence of a suspected fetal skin disorder (Herlitz syndrome, epidermolysis bullosa atrophicans generalisata gravis, in two cases; bullous ichthyosiform erythroderma in the other two). The remaining 14 women were to undergo elective abortion by hysterotomy. The study was approved

by the Ethics Committee of the University Hospital of Lund. The procedure was explained to the women and their consent was obtained. The positions of the placenta and fetus were determined by ultrasound.

In the first 13 women the biopsy was taken "blind", and in the remaining five it was obtained under direct vision by a two-cannula procedure.

The "blind" biopsy technique. A sharp trocar and cannula (diameter 2.2 mm) were introduced percutaneously into the amniotic cavity under either local or general anesthesia. The trocar was withdrawn and a "Needlescope" (Dyonics, Inc.), 1.7 mm in diameter, was inserted through the cannula. A site suitable for biopsy of the skin was chosen (back, buttock, or thigh). The cannula was placed gently on the fetal skin at the selected site. The fetoscope was withdrawn, a biopsy forceps (2×2 mm jaws) was passed down the cannula and a biopsy specimen (about 1 mm in diameter) was obtained "blind".

The two-cannula biopsy technique. Under general anesthesia, the uterus was exposed by a low transverse abdominal incision (Pfannenstiel). The trocar and cannula were introduced through the myometrium into the amniotic cavity.

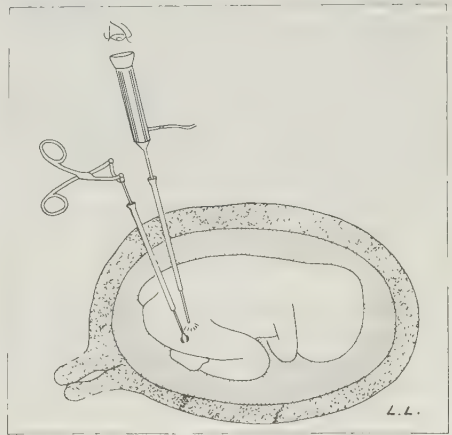


Fig. 1. Fetal skin sampling under direct vision by the two-cannula technique.

Table 1. *Fetal skin sampling.*

Case No. #	Gestational age (weeks)	No. of biopsies	Tissue obtained			
			Skin	Fetal membranes†	Myometrium††	Trophoblast††
“Blind” biopsy						
1	17	3	0	2	1	0
2	21	5	1	2	0	2
3	16	3	0	2	1	0
4	21	5	0	3	1	1
5	21	1	1	0	0	0
6*	19	8	6	2	0	0
7*	20	10	7	0	1	2
8	19	8	2	4	2	0
9	17	1	0	1	0	0
10	16	6	2	3	0	1
11	16	8	1	6	1	0
12*	19	8	2	3	2	1
13	20	5	2	3	0	0
Total		71	24	31	9	7
Biopsy under direct vision						
14	17	1	1	0	0	0
15	17	8	8	0	0	0
16	17	6	6	0	0	0
17	18	2	2	0	0	0
18*	20	3	3	0	0	0
Total		20	20	0	0	0

Cases numbered in the order in which they were studied. † This specimen consisted of either amnion alone or both amnion and chorion.

†† In most cases myometrial specimens included fetal membranes. * Diagnostic fetoscopy (see Results).

The trocar was then removed and a Needlescope inserted through the cannula. A site suitable for skin biopsy was selected (thigh in cases 14–16; scalp in cases 17 and 18). A second trocar and cannula were introduced into the amniotic cavity 3–5 cm from the site of insertion of the first Needlescope. The trocar was withdrawn and a second Needlescope was passed down and visually guided towards the selected site. The second Needlescope was then replaced with a biopsy forceps (2×2 mm jaws), and biopsy specimens were taken under direct vision.

Microscopy. The specimens were immediately placed in fixative solution. Those from the first five cases for elective abortion and from the four diagnostic cases were sent to Heidelberg, where they were prepared for light and electron microscopy as described elsewhere (1). The samples from the remaining cases were processed in Lund; they were embedded in paraffin wax, sectioned, stained with eosin-hematoxylin and by van Gieson's method, and then examined in a light microscope.

RESULTS

The results are presented in the Table. Thus, out of 71 biopsy specimens taken "blind", only 24 consisted of skin, while the remaining 47 were either fetal membranes, myometrium or trophoblastic tissue. All 20 biopsy specimens taken by the two-cannula technique consisted of skin.

Diagnostic cases. Of the four women admitted for the purpose of prenatal diagnosis, the "blind" biopsy procedure was used in the first three, and the two-cannula procedure in the fourth. In the first case (No. 6 in the Table), the Herlitz syndrome, i.e., epidermolysis bullosa atrophicans generalisata gravis, could be excluded (1, 7). The pregnancy proceeded uneventfully, and in the 41st week of gestation a healthy boy was born. Examination of the infant's skin revealed no scars or sequelae at the sites of fetal biopsies. In the second diagnostic case (No. 7 in the Table), bullous ichthyosiform erythroderma was diagnosed, and the pregnancy was interrupted by hysterotomy. Examination of skin samples from the abortus confirmed the diagnosis. In the third case (No. 12), the Herlitz syndrome was excluded. There were no early complications after the fetoscopy, but from the 26th week of gestation the woman was hospitalized because of intermittent leakage of amniotic fluid. Labor started in the 33rd week, and a healthy boy weighing 2.02 kg was delivered by cesarean section. No scars were seen at the sites of fetal biopsy, and no signs of any skin disorder were found. In the fourth diagnostic case (No. 18), where the two-cannula biopsy procedure

was used, bullous ichthyosiform erythroderma was excluded. To date, 7 weeks after the fetoscopy, this pregnancy is continuing normally.

DISCUSSION

Rodeck et al. (8) used a specially constructed biopsy forceps which is so tiny that it can be passed through the cannula together with the fetoscope, thereby permitting biopsy of the skin under direct vision with use of only one cannula. This tiny forceps is not commercially available. So far, we and others have had to rely on "blind" biopsy.

The "blind" biopsy procedure may be complicated by inadvertent slipping of the tip of the cannula off the fetus and onto the fetal membranes lining the uterine wall or lining the placenta, from which the biopsy specimen is then removed. Using the "blind" technique in a case for prenatal diagnosis, Elias et al. (4) obtained only one single biopsy specimen and even that turned out to be a piece of amniotic membrane; four weeks later, they repeated the fetoscopy with success. In the present series, specimens obtained by the "blind" method consisted of skin in about only one out of every three cases. The remainder comprised fetal membranes, myometrium or trophoblast. In one of our diagnostic cases (No. 12 in the Table), where the "blind" technique was used, six of the eight biopsy specimens obtained were from tissue other than skin and the damage to the amniotic sac caused by such unsuccessful biopsy attempts was probably responsible for the intermittent leakage of amniotic fluid that occurred from the 26th week of gestation and for the premature delivery in the 33rd week.

Thus, there is an urgent need for a technique that makes it possible to perform the biopsy under direct vision. In this paper we have described the use of such a technique, the two-cannula method. Although it has proved practicable, with this method the uterine wall has to be perforated twice with concomitant increased risk of miscarriage. We would certainly prefer a one-cannula approach if such were available.

ADDENDUM

Since this manuscript was submitted for publication, patient No. 18 gave birth to a normal infant. When she was 20 weeks pregnant we had sampled fetal skin specimens under

direct vision by means of the two-cannula technique. The pregnancy then continued uneventfully to parturition in the 41st week.

At the International Meeting on Fetoscopy in Fetal Diagnosis and Therapy in San Francisco, October 15–17, 1982, 45 diagnostic cases of fetal skin sampling were reported from altogether 11 centres. With the exception of the Rodeck Centre at King's College Hospital, London and, as regards our last few cases, the Department of Obstetrics and Gynecology at Lund University Hospital, all centres used the "blind" biopsy procedure. In 14 of the 45 cases, the fetus was affected and the pregnancy had to be terminated. Of the 31 women who continued with their pregnancy, 6 were still undelivered. Among the remaining 25 pregnancies, there were 4 miscarriages (16%), all occurring after "blind" biopsy.

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Submitted for publication July 29, 1982.

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“Blind” versus direct vision technique for fetal skin sampling in cases for prenatal diagnosis

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Seven fetuses at risk of developing a serious inherited skin disorder (epidermolysis bullosa atrophicans generalisata gravis in 4, bullous ichthyosiform erythroderma in 2, and non-bullous ichthyosiform erythroderma in 1) were subjected to prenatal diagnosis by fetal skin sampling. The conventional “blind” biopsy procedure was used in the first 3 cases; a two-cannula technique (one cannula for the optic instrument and the other for the biopsy forceps) that permits biopsy of the skin under direct vision, was employed in the remaining 4 cases. With the “blind” technique, 8 to 10 biopsy specimens had to be taken to ensure that enough skin material would be available for the microscopic examination; only one specimen out of every two was found to consist of skin; the remainder comprised fetal membranes, myometrium, or trophoblast. In one case where the “blind” procedure had been used, leakage of amniotic fluid occurred and labor started in the 33rd week. With the two-cannula technique, the number of biopsy samples could be confined to two or three, and all proved to be of skin.

Received 28 June, accepted for publication 23 September 1983

Key words: Biopsy; bullous skin diseases; fetoscopy; fetus; prenatal diagnosis.

In the April 1980 issue of *Clinical Genetics*, Elias and co-workers reported the first successful fetal skin sampling in utero in a case for prenatal diagnosis (Elias et al. 1980). Elias took the skin specimens “blind”, a technique that has since become the conventional method for fetal skin sampling. One disadvantage of the procedure is that tissue specimens may be inadvertently removed from fetal membranes, the myometrium, or even the trophoblast (Löfberg & Gustavii 1982). Elias, in his first attempt, obtained solely a piece of the amniotic membrane and thus no skin specimen; (4 weeks later, he repeated the fetoscopy with success). The damage caused by unsuccessful biopsies may induce abortion or premature birth (Löfberg & Gustavii 1982).

In a previous paper we presented a technique that enables biopsy of the skin to be effected under direct vision by means of two cannulas, one for the optic instrument and the other for the biopsy forceps (Löfberg & Gustavii 1982). Here we report a series of 7 diagnostic cases; the “blind” technique was used in the first 3 and the two-cannula technique in the remaining 4.

Material and Methods

Clinical material

The clinical material included 7 cases of skin sampling from the fetuses of 6 women (Table 1). In one of the women, the examination was done in two consecutive pregnancies (cases 2 and 4 in the Table). In the

Table 1
Diagnostic cases of fetal skin sampling

Case No. *	Gestational age (weeks)	Genetic risk	Tissue obtained					Fetal diagnosis	Outcome	
			No. of biopsies	Skin	Fetal membranes†	Myometrium‡	Trophoblast‡			
"Blind" biopsy										
1	19	Herlitz syndrome§	8	6	2	0	0	Normal	Term delivery - normal newborn skin	
2	20	Bullous ECI§	10	7	0	1	2	Bullous ECI	Bullous ECI in abortus	
3	19	Herlitz syndrome	8	2	3	2	1	Normal	Premature delivery (33rd week) - normal newborn skin	
Biopsy under direct vision										
4	20	Bullous ECI	3	3	0	0	0	Normal	Term delivery - normal newborn skin	
5	19	Herlitz syndrome	2	2	0	0	0	Normal	Term delivery - normal newborn skin	
6	19	Herlitz syndrome	2	2	0	0	0	Normal	Term delivery - normal newborn skin	
7	21	Non-bullous ECI	2	2	0	0	0	Normal	Undelivered	

* Cases numbered in order in which they were studied.

† This specimen consisted of either amnion alone or both amnion and chorion.

‡ In most cases this specimen included fetal membranes.

§ Herlitz syndrome epidermolysis bullosa atrophicans generalisata gravis epidermolysis bullosa letalis.

§ ECI=congenital ichthyosiform erythroderma.

first 3 cases, the skin biopsies were taken "blind"; in the remaining 4, they were obtained by a two-cannula technique that permits sampling of the skin under direct vision.

Case 1: The woman had one healthy boy and a girl who suffered from the Herlitz syndrome (epidermolysis bullosa atrophicans generalisata gravis = epidermolysis bullosa letalis) and who died from sepsis at the age of 19 days. Both parents must be regarded as heterozygous carriers of the Herlitz gene, which is transmitted in an autosomal recessive pattern. The parents were informed of the 1:4 risk of their having a child affected by the disease. In a subsequent pregnancy the woman requested prenatal diagnosis. In the 19th week, fetal skin specimens were obtained at fetoscopy.

Cases 2 and 4: The woman herself suffers from bullous congenital ichthyosiform erythroderma (bullous ECI). From the first day of her life, serous blisters and patchy superficial epidermolysis had appeared all over her body. New bullae appeared almost daily in her first decade. Due to secondary infections, she had been frequently hospitalized, especially in childhood. As an adult, she had blister eruptions, with free intervals of only a few weeks. During her first year of marriage, the woman avoided becoming pregnant because of her fear of having an affected child, the risk of which is 1:2. Then, after having received genetic counselling, she and her husband considered pregnancy, but only because of the availability of prenatal diagnosis of the disorder. In two consecutive pregnancies, fetal skin specimens were obtained at fetoscopy, which, in both cases, was carried out in the 20th week of pregnancy.

Cases 3, 5 and 6: These cases resemble case 1, with one baby of each woman suffering

from Herlitz syndrome and succumbing at the age of 2 to 5 months. In each of these cases, in the 19th week of a subsequent pregnancy, fetal skin specimens were taken at fetoscopy.

Case 7: The couple had a son with non-bullous congenital ichthyosiform erythroderma (non-bullous ECI). They felt that they should not have a second affected child, and the woman had avoided becoming pregnant. However, after having been informed of the possibility of prenatal diagnosis the couple considered another pregnancy. In the 21st week of that pregnancy, fetal skin specimens were taken at fetoscopy.

Fetal skin sampling

The "blind" biopsy technique: A sharp trocar and cannula (diameter 2.2 mm) were introduced percutaneously into the amniotic cavity under local anesthesia. The trocar was withdrawn and a "Needlescope" (Dyonics, Inc.), 1.7 mm in diameter, was inserted through the cannula. A site suitable for skin biopsy was chosen. The cannula was placed gently on the fetal skin at the selected site. The fetoscope was withdrawn, a biopsy forceps (2 × 2 mm jaws) was then passed down the cannula and biopsy specimens were obtained "blind".

The two-cannula biopsy technique: Under general anesthesia the uterus was exposed by a low abdominal incision. (This has become the routine approach at diagnostic fetoscopy at our department (Gustavii et al. 1983)). A sharp trocar and cannula were introduced through the myometrium into the amniotic cavity. The trocar was removed and a Needlescope was inserted through the cannula. A site suitable for biopsy of the skin was selected. A second trocar and cannula were then introduced into the amniotic cavity, 3–5 cm from the site of insertion of the first Needlescope. The

trocar was withdrawn and a second Needle-scope was passed down and guided visually towards the selected site. The second Needlescope was then replaced with a biopsy forceps, and biopsy specimens were taken under direct vision.

Site of biopsy: Since having introduced the two-cannula technique, we have examined 2 fetuses at risk for ichthyosiform erythroderma (cases 4 and 7 in the table). In these 2 cases, the biopsies were taken from the scalp. In the remaining 5 cases, the sites chosen for biopsy were the thigh, hip, buttock, back, or shoulder.

Microscopy

The specimens were immediately placed in fixative solution. They were sent to Heidelberg and there processed and examined under the electron microscope by Professor Ingrid Anton-Lamprecht (Anton-Lamprecht 1981).

Results

The results are presented in Table 1. Of the 26 biopsy specimens taken "blind", only 15 consisted of skin. Of the remaining 11 specimens, 5 were fetal membranes; 3, myometrium; and 3, trophoblastic tissue. By contrast, all specimens taken under direct vision by the two-cannula technique were skin.

On the basis of the ultrastructural examination, one of the fetuses at risk of having bullous ichthyosiform erythroderma (case 2 in the Table) was found to be affected. In the remaining 6 fetuses at risk, the disorder was excluded.

The pregnancy of the affected fetus was terminated by hysterotomy (Dr. Kåre Molne, Rikshospitalet, Oslo). The 480 g fetus had skin lesions on wrist and hyperkeratosis on scalp, face, palms, and soles. Barely visible punctate changes in the skin on the right hip and the right buttock

suggested the sites of the fetoscopy biopsies carried out 16 days previously. Light and electron microscopy of skin specimens from the abortion confirmed the diagnosis of bullous ichthyosiform erythroderma.

Four of the six pregnancies with unaffected fetuses proceeded uneventfully to parturition at term; at this writing one woman is still pregnant. In the remaining case (case 3 in the Table), where the "blind" technique was used, the woman was hospitalized from the 26th week of gestation because of intermittent leakage of amniotic fluid. Labor started in the 33rd week, and a healthy boy weighing 2.02 kg was delivered by cesarean section. In this case, only 2 of the 8 biopsies taken were skin; the remaining 6 were fetal membranes in 3 of the cases, myometrium in 2, and trophoblastic tissue in 1.

The 5 children are developing normally, the oldest one now being 2 years 5 months old. Barely visible scars, suggesting the sites of biopsy, were found in only one of the newborns (case 5 in the Table); one scar was found in the right shoulder region, another on the upper back.

The time needed for processing of the biopsies and for diagnostic electron microscopy was, in the cases at risk of Herlitz syndrome, 7–10 days; in the cases at risk of ichthyosiform erythroderma, 11–13 days.

Discussion

The "blind" biopsy procedure has several serious drawbacks. First, specimens of tissues other than skin may be inadvertently removed if the tip of the cannula slips off the fetus and onto the uterine wall or the placenta. Injury resulting from such unsuccessful biopsies can cause miscarriage or premature birth. Second, several biopsy specimens may have to be taken to ensure that enough skin material will be available for the microscopic examination. Third, due to the risk

of injury to the face (eyes, lips, nose, etc.), the "blind" method is unsuitable for biopsy attempts from the scalp, the site where the first ultrastructural signs of keratinization disturbance ought to appear, according to the work of Holbrook & Odland (1980) on normal fetal skin development.

However, the two-cannula technique (one cannula for the optic instrument and the other for the biopsy forceps) permits biopsy of the skin under direct vision. With this method, used in the last four fetoscopies of the present study, we were able not only to take the biopsy specimens from the scalp in 2 cases at risk for ichthyosiform erythroderma but also to confine the number of biopsies to two or three.

To the best of our knowledge, the only previous report on fetal skin sampling under direct vision is that of Rodeck (Rodeck et al. 1980). He used a specially constructed biopsy forceps which is so tiny that it can be passed through the cannula together with the fetoscope. But this tiny forceps is not commercially available. However, the instruments used in the two-cannula procedure presented in this report are all available in the open market.

In the 4 cases where the two-cannula technique was used, 3 women gave birth at term after an uneventful pregnancy; the

remaining woman is now in her 36th week of gestation, and the pregnancy is continuing normally.

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FETAL MUSCLE BIOPSY

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Abstract. Fetal muscle specimens suitable for histological and histochemical studies were obtained under fetoscopic visualization by removing a small piece of skin, thereby exposing the underlying muscle. The procedure was carried out in 7 cases prior to second-trimester abortion. Muscle specimens so obtained may become useful in the prenatal diagnosis of Duchenne muscular dystrophy.

No reliable *in utero* test for inherited muscular dystrophies is available. In the commonest and most severe of these disorders, Duchenne muscular dystrophy, measurement of creatine phosphokinase activity in fetal serum obtained at fetoscopy in the second trimester of high-risk pregnancies has been used as a diagnostic test. However, Emery (5) and Golbus (6) and their co-workers found that the disease was not manifested in all affected cases by elevated creatine phosphokinase activity in the second trimester. It is therefore *not* recommended that Duchenne muscular dystrophy be diagnosed solely on the basis of fetal serum creatine phosphokinase activity.

On the other hand, examination of fetal muscle obtained *ex utero* from male fetuses at risk and therapeutically aborted in the second trimester has revealed histological and histochemical alterations compatible with Duchenne muscular dystrophy (2–5, 11, 12).

We describe here a technique for obtaining biopsy specimens of fetal muscle *in utero*. The specimens obtained were examined by those histological and histochemical methods that are routinely used to diagnose Duchenne muscular dystrophy.

CLINICAL MATERIAL AND METHODS

The fetuses of 7 women in the 16th to 20th week of gestation (menstrual weeks) were studied. The women were scheduled to undergo elective abortion by abdominal hysterotomy.

There was no history of any neuromuscular disorder in these cases. The Ethical Committee of Lund University Hospital had approved the study and informed consent was obtained from the patient. The position of the placenta and that of the fetus were determined by ultrasound.

Under general anesthesia, the uterus was exposed by a low abdominal incision (Fig. 1). (This has become the routine approach at diagnostic fetoscopy at our department.) A sharp trocar and cannula (diameter 2.2 mm) were introduced through the myometrium into the amniotic cavity. The trocar was removed and a 1.7 mm diameter Needlescope (Dyonics, Inc.) was inserted through the cannula. A site suitable for muscle biopsy was chosen (thigh or calf). A second trocar and cannula were then introduced into the amniotic cavity about 3–5 cm from the insertion site of the first Needlescope. The trocar was withdrawn and a second Needlescope was passed down and guided by direct vision towards the selected site. The second Needlescope was then replaced by a biopsy forceps (2 x 2 mm jaws). Under direct vision, a small piece of skin was removed and two biopsy specimens were obtained from the underlying exposed muscle. The specimens were immediately dipped in talcum, frozen in liquid nitrogen, and stored at -70°C until analysed.

The following stains and enzyme reactions were employed.



Fig. 1. Open abdomen fetoscopy. Drawing by Louise Goldberg, Lund University Hospital.

ed: hematoxylin-eosin, van Gieson, myofibrillar ATPase at pH 9.4 and 4.6, and alizarin red S. Fiber diameter and fiber area were determined by a digitizer connected to a calculator (Sandstedt 1981).

RESULTS

Examination by microscopy of the fourteen biopsy specimens from the seven fetuses revealed that all contained muscle fibers. Each measured about 0.5 to 1.0 mm across and contained 300 to 900 fibers (Fig. 2). Mean fiber diameter was $9.9 \pm 1.1 \mu\text{m}$ (range 8.6–12.1) and mean fiber area $110 \pm 18 \mu\text{m}^2$ (range 90–140).

The material was sufficiently large and well-preserved to allow application of those stainings and enzyme reactions attempted (see Methods). No eosinophilic or hyaline fibers and no calcium-positive fibers were identified.

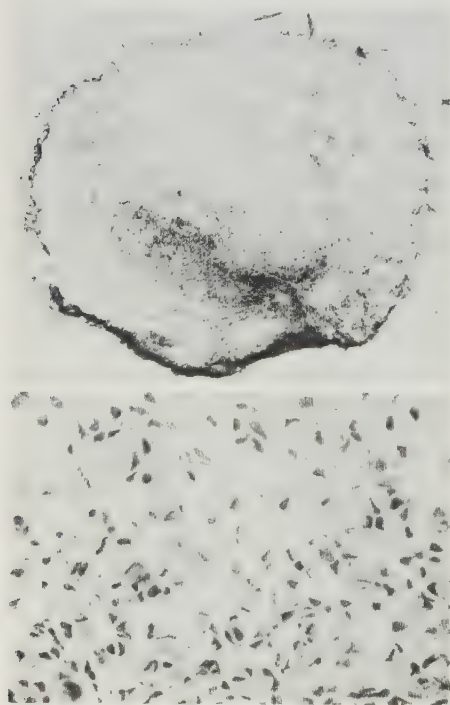


Fig. 2. Cross-section of normal thigh muscle specimen obtained *in utero* at fetoscopy from a fetus in the 17th week of gestation. (a) Section of the whole biopsy specimen ($\times 50$); (b) the same section at higher magnification ($\times 315$). H.-E.

Post-abortem examination of the biopsy site showed that the diameter of the skin lesions was about 2 mm; the muscle lesions were barely visible.

DISCUSSION

With this technique it has proved possible to obtain biopsies of fetal muscle. The specimens (diameter about 0.5 to 1.0 mm) were taken from large muscles, such as thigh or calf. Wounds from surgery *in utero* heal rapidly (7). Skin biopsies performed on the fetus in the second trimester appear not to leave any scars or sequelae visible after birth (8). It has also been shown that muscle tissue has a great capacity to regenerate (1).

The sampling was carried out with the uterus exposed by a low abdominal incision. This has become the routine approach at fetoscopy at our department. Open abdomen fetoscopy has several advantages. First, the fetoscope can be inserted leaving a good margin to the large myometrial vessels, damage to which can cause bleeding into the amniotic cavity, thereby impairing vision and making sampling difficult or impossible. Second, penetration of the leathery amniotic membrane is facilitated by the ability to insert the fetoscope exactly perpendicular to the amniotic sac and to apply the thrust at the right moment. Third, in cases of anterior placenta, the fetoscope can be introduced through the posterior uterine wall, thereby avoiding damage to the placenta. Fourth, insertion of the fetoscope can be done more precisely over the site that, judged by ultrasound, seems to be the most suitable one for the sampling. Fifth, the woman is caused less discomfort when under general anesthesia than when undergoing fetoscopy under local anesthesia.

With the exception of the work by Brambati et al. (2), previous investigations of muscle from *normal* fetuses have included some necropsies from prostaglandin or saline abortuses (Table I). It is questionable if values obtained from such specimens can be used as a reference. In the work by Brambati et al. (2), whole (tendon-to-tendon) muscles of hysterotomy abortuses were removed and immediately frozen. In such preparations, reliable values of fiber diameter and variation in fiber diameter can be obtained. Nevertheless, these data are not suitable for comparison with those from small biopsies, such as fetoscopy samples, since whole-muscle preparations are easier to handle (so avoiding contractions) than are small biopsy specimens (contractions affect both the diameter of the fibers and their stainability). In the present study, the

Table 1. Results obtained in muscle from normal fetuses.

Authors	No. of cases	Method of termination	Muscle examined	Fiber diameter (mean±SD in μm)
Winn & Heller (12)	2	Saline	Gastrocnemius, biceps femoris	3.8±0.7
Mahoney et al. (9)	10	Prostaglandin	Deltoid	6.9±1.7
			Quadriceps	5.3±1.2
			Gastrocnemius	4.4±0.9
Emery et al. (4,5)	16	Prostaglandin or hysterotomy	Quadriceps	6.9–10.7*
Brambati et al. (2)	5	Hysterotomy	Quadriceps, biceps femoris	7.9±0.9

*Range with variance 0.8–3.6.

mean fiber diameter ($9.9\pm 1.1\ \mu\text{m}$) obtained from fetoscopy samples was found to be larger than that obtained from muscles of post-abortem fetuses (Table 1).

In the second-trimester fetuses affected by Duchenne muscular dystrophy, muscle pathology is not characterized by muscle fiber necrosis, muscle fiber regeneration, or an increased amount of fat or connective tissue, as is the case in affected boys (3, 12). In fetuses, the diagnosis has to be based on indices such as increased fiber size, increased variation in fiber size, and increased proportions of eosinophilic, hyaline, and calcium-positive fibers (2–5, 11, 12).

In two at-risk fetuses presumed to be affected, Emery & Burt (4) found that the content of eosinophilic fibers was 6.9–8.0 per cent and that of calcium-positive fibers (alizarin stain) 8.4–12.0 per cent. In a similar study of two other such fetuses, Brambati and co-workers (2) found 3.5–4.3 per cent hyaline fibers and 3.8–4.8 per cent fibers containing calcium. Histopathological changes occurring in that range ought to be detectable in biopsy specimens of the size obtained in the present study.

We have thus presented a technique suitable for fetal muscle biopsy. At present, this method is not recommended for clinical use. Before such use, the reference values for fetoscopy samples obtained from normal fetuses in the present study have to be compared with those of fetoscopy samples obtained from at-risk fetuses of women who have decided to terminate their pregnancy.

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Submitted for publication December 21, 1982

Accepted July 8, 1983

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First-trimester diagnosis on chorionic villi obtained by direct vision technique

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Summary. An improved technique for direct vision chorionic biopsy that gives a clear view of the amniotic sac was developed. With this technique, used in 48 women prior to vacuum aspiration and in six cases for diagnosis (karyotyping or enzyme analysis), it was possible to obtain chorionic villi free from contamination by maternal tissue. It was also possible to pick out villi (rich in blood vessels and with abundant buds on their surface) found to be most capable of growing in vitro. In the diagnostic cases, the pregnancies have continued uneventfully since the sampling; one pregnancy is now in the 32nd week.

Introduction

Simoni et al. (1983) tested various approaches for the sampling of chorionic villi. They concluded that the technique of *blind* catheter aspiration presented by Old et al. (1982) might be the best method. With the blind technique, however, up to four aspiration attempts may have to be made to obtain adequate material (Old et al. 1982; Pergament et al. 1983), and in the study by Simoni et al. (1983) from 30 min up to 1 h was needed to separate villi from maternal decidual fragments. Attempts at separation may not always be successful or the results reliable (Tietung 1975; Niazi et al. 1981).

In the present study we have used a modification of the *direct vision* technique (Kullander and Sandahl 1973; Gustavii 1983) that gives a clear view of the amniotic sac and enables chorionic villi to be taken without contamination with maternal tissue. Here we report a series of six diagnostic cases using this technique.

Material and methods

Preliminary studies

Preliminary studies were carried out on 54 women who were to undergo first-trimester termination of pregnancy. In 48 of them, chorionic biopsy samples were taken; in the remaining six controls, intra-uterine pressure was recorded during instillation of known amounts of saline into the extra-amniotic space.

Clinical material

The clinical material included six women in the 8th to 12th week (menstrual weeks) of gestation. Table 1 shows the indica-

tions for sampling. Case 1 is presented in greater detail as difficulties were encountered in establishing the diagnosis.

Case 1. The woman already has one healthy boy and one boy who suffers from metachromatic leukodystrophy. She had also undergone a late saline abortion (22nd week) of an affected fetus diagnosed using cultivated amniotic fluid cells. In a subsequent twin pregnancy the woman requested prenatal diagnosis by chorionic biopsy. In the 8th week, specimens of chorionic villi were taken (Fig. 1) for determination of arylsulphatase A activity.

Anesthesia

Local anesthesia was used in four of the cases and general anesthesia in the remainder, including those for diagnosis.

Sampling technique

We use a specially constructed cannula (Richards Scandinavia AB) with an operating channel, 3.0 × 4.7 mm in cross-section, and two attachments, one for the biopsy forceps and the other for instillation of body-warm saline solution (Fig. 2) (Gustavii 1983). With the Needlescope (Dyomics, Inc., 1.7 mm in diameter) in position in the cannula and the saline "drip" functioning, the set of instruments is carefully introduced through the cervical canal. On reaching the internal orifice, the extra-amniotic space is already distended by the continuously instilled saline, and the ovum is instantly and clearly visualized. The biopsy forceps are introduced. From the base of the amniotic sac, a fragment of decidua is removed. The biopsy forceps are withdrawn, cleaned from decidual fragments, reinserted, and samples of villi are taken. At first we took extra-placental villi adjacent to the implantation site; we now take specimens of placental villi 2–3 cm from the edge of the placenta. The specimens are examined immediately under a dissecting microscope in the operating room to ascertain that only villi have been removed. The Needlescope is withdrawn, allowing saline to flow out through the cannula.

Ultrasound

Fetal heart activity is determined by ultrasound before and after the procedure. In the preliminary studies, ultrasound scans were made to estimate the degree of uterine distension.

Intra-uterine pressure recording

Intra-uterine pressure recording was made with a Gaeltec microtransducer catheter introduced through a Foley catheter

Table 1. Diagnostic cases of chorionic biopsy

Case No. ^a	Maternal age (years)	Gestational age at sampling (weeks)	Genetic risk/ Indication for sampling	Analysis	Fetal diagnosis	Time needed for diagnosis (days)	Gestational age to date (weeks)
1	34	8	Previous child with metachromatic leukodystrophy	Enzyme activity	None (see text)		32
2	45	11	Previous late termination of pregnancy with Mb Down fetus	Karyotype	Normal (46,XY)	14	22
3	18	12	Hemophilia A	Sex	Male (46,XY)	16 ^b	21 ^c
4	29	9	Hemophilia A	Sex	Male (46,XY)	23 ^b	16 ^d
5	41	10	Maternal age	Karyotype	Normal (46,XY)	13	15
6	38	9	Previous child with Mb Down	Karyotype	Normal (46,XY)	15	13

^a Cases numbered in chronologic order
^b Time needed for sexing by chromosomal analysis; sexing by X- and Y-chromatin examination on direct preparations was completed within 1–2 h of sampling
^c After having seen the fetus repeatedly at ultrasound examination, the woman decided to continue her pregnancy without undergoing fetal blood sampling by fetoscopy for factor VIII determination
^d Fetal blood sampling for factor VIII determination scheduled for the 19th week



Fig. 1. Ultrasound scan made in the eighth week of a twin pregnancy; our first case for diagnosis. *Small arrows* indicate biopsy sites. *Large arrow* points to the narrow space between the uterine wall and the amniotic sacs

located in the extra-amniotic space; saline being instilled through the catheter.

Sex determination and chromosome analysis

The specimen is placed in culture medium, transported to the laboratory and there processed within 30 min of sampling. The medium is discarded and a minimal piece of the specimen is immediately used to prepare two slides for X- and Y-chromatin screening according to conventional methods. The remaining portion of the specimen is minced with scissors and the pieces are distributed into a Flascette chamber, incubated with 3 ml minimum essential Eagle medium with Earle's salts, supplemented with 20% fetal calf serum and 10% human serum. The

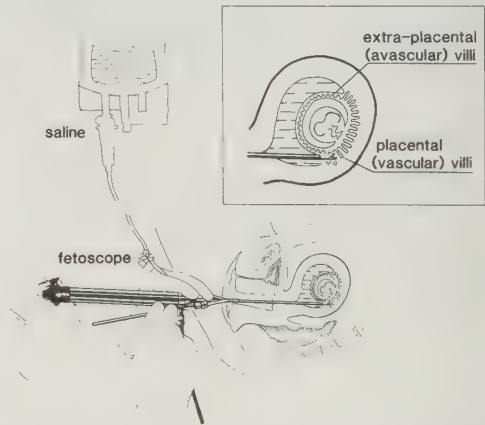


Fig. 2. Sampling of placental villi during instillation of saline which gives a clear view of the biopsy site and enables direct vision sampling of vascular villi. Drawing by Ingemar Nilsson

culture is incubated at 37° C in 5% CO₂ in air. When colonies of actively dividing cells are observed, colchicine (final concentration 0.01 µg/ml) is added for 16 h. The plastic chamber and gasket are removed and the glass slide dipped into 100 ml 0.075 mol/l KCl for 30 min, and 20 ml fixative (methanol : acetic acid, 3 : 1) is added. After 5 min, 30 ml of the solution is replaced with an equal amount of fresh fixative. This procedure is repeated twice with 50 ml and 100 ml fixative, respectively. The slide is finally fixed for a further 30 min in fresh fixative, air-dried at room temperature, and stained according to the trypsin-Giemsa banding technique. As a routine, 11 well-spread metaphases from different colonies are analyzed.

Table 2. Amount of chorionic tissue in which enzyme activity was detectable in normal pregnancies (therapeutic abortions)

Disease	Enzyme analyzed	Amount of tissue (mg) ^a
<i>Glycoproteinoses</i>		
α -Mannosidosis	α -Mannosidase	1
" β -Mannosidosis"	β -Mannosidase	1
Fucosidosis	α -Fucosidase	1
<i>Sphingolipidoses</i>		
GM ₁ Gangliosidosis	β -Galactosidase	1
GM ₂ Gangliosidosis		
Type I, Tay-Sachs	β -Hexosaminidase A	1
Type II, Sandhoff	β -Hexosaminidase A + B	1
Metachromatic leukodystrophy	Arylsulfatase A	5
<i>Mucopolysaccharidoses</i>		
I Hürler, Scheie	α -Iduronidase	5
II Hunter	Iduronide sulfatase	5
IIIA Sanfilippo A	Sulfamatase	Not detectable ^b
IIIB Sanfilippo B	α -N-Acetylglucosaminidase	1
IIIC Sanfilippo C	N-Acetyltransferase	1
IV Morquio	Galactose-6-sulfatase	Not detectable ^b
VI Maroteaux-Lamy	Arylsulfatase B	5
VII β -Glucuronidase deficiency	β -Glucuronidase	1

^a One, 5, and 10 mg of tissue obtained by chorionic biopsy was examined^b Not even in 100 mg of tissue obtained from vacuum aspirated placentas was any activity detectable

Methods for enzyme determination

The chorionic biopsy specimen is placed immediately in 1 ml cold 0.9% NaCl, transported to the laboratory, and processed within 30 min of sampling. After homogenising the specimen, a sample is removed for protein analysis and the remainder used immediately for enzyme analysis. Glycosidases were analyzed using methylumbelliferyl glycosides. Sulfatases were analyzed as described previously (Hall et al. 1978). Acetyltransferase was analyzed according to the method of Hopwood and Elliott (1981).

Results

Fetal heart activity after the procedure was the same as before, i.e., normal. The amount of saline needed to distend the extra-amniotic space, as calculated from the scans, was at most 150 ml. The increase in intra-uterine pressure after instillation of 150 ml saline in the controls was 15 to 20 mm Hg, i.e., in the same range as that of the ordinarily painless Braxton Hicks contractions normally experienced from early stages of gestation. The four women undergoing the procedure under local anesthesia experienced no discomfort.

Attempts to cultivate extra-placental villi (destined to degenerate) usually failed. However, cells of placental villi, which are rich in blood vessels, grew and analyzable metaphase plates were obtained. Extra-placental villi, which are technically more simple to obtain than placental villi, were useful for enzyme determination (Table 2), and for sexing by X- and Y-chromatin examination which could be done on one single villus.

Diagnostic cases

Case 1. No activity of arylsulfatase A was detectable in the specimens of the twin pregnancy or in those of the controls. The reference enzyme β -hexosaminidase had normal activity. Simoni et al. (1983) were also unable to detect arylsulfatase A activity in chorionic villi from a normal pregnancy (therapeutic abortion). Our later studies showed that 5 mg tissue is needed for determination of arylsulfatase A activity; in the clinical case the weight of each specimen obtained was about 1 mg. Since diagnosis by chorionic biopsy was unsuccessful, the woman continued her pregnancy and amniocenteses (one of each gestational sac) were performed in the 17th week. Enzyme activity determination on cultured amniotic fluid cells showed that one fetus was unaffected and the other affected. (Cells also analyzed by Prof. L. Svennerholm, Gothenburg). In the 22nd week, a "selective abortion" was performed, as described by Åberg et al. (1978). The woman is now in her 32nd week of gestation, and the pregnancy is continuing normally.

Table 1 shows the results of the remaining diagnostic cases. Vaginal bleeding was usually observed for a few days after the sampling. No other side effects were experienced, and the pregnancies have so far continued normally after the chorionic biopsy.

Discussion

With this technique contamination with maternal tissue is easily avoided. It is possible even to pick out by direct vision such villi (see below) proved to be those most capable of growing in vitro. A single villus or up to at least 10 mg of tissue can be

removed as required. Of those enzymes studied (Table 2) sulfamatase and galactose-6-sulfatase were undetectable in 10 mg chorionic biopsy specimens and even in 100 mg tissue obtained from vacuum aspirated placentas. It cannot be taken for granted that all enzymes are being produced in chorionic tissue already in the first trimester.

Our observation that extra-placental villi are difficult to culture is in agreement with the finding of Kullander and Sandahl (1973) who in 20 out of 41 cases were unable to induce such villi to grow in vitro. Most extra-placental villi from about the 9th week of pregnancy were found to be enlarged, edematous, and containing few or no vessels and with no buds on their surface. In contrast, placental villi taken 2-3 cm from the edge of the placenta were thin, rich in blood vessels, and with abundant buds. These villi grew in vitro and analyzable metaphase plates were obtained within 2-3 weeks.

In the six diagnostic cases of the present study, there have been no obstetric complications so far. In a report from China (Tietung 1975) on blind catheter aspiration for fetal sexing, four miscarriages (5.7%) resulted among the 66 continuing pregnancies. G. Simoni and B. Brambati (personal communication) noted two miscarriages (about 7%) among 29 such pregnancies. In one of the two cases of miscarriage, chorionic biopsy examination revealed a low mitotic index such as occasionally seen in spontaneous abortion.

The frequency of spontaneous abortion toward the end of the first trimester is difficult to assess as the number of women aborting at home and not attending hospital is unknown. Attempts to make such an estimation are also hampered by the fact that (in Sweden) one pregnancy out of every four is terminated legally. At the Department of Obstetrics and Gynecology of Lund University Hospital, during the years 1980 and 1981, 393 women were treated for spontaneous or missed abortion in the 12th week or later; during the same period of time 5,938 women gave birth. A rough estimation, on the basis of these figures and with the above reservations, suggests that a woman in the 12th week runs a 6.2% risk of aborting spontaneously; a frequency that is not appreciably different from

the frequency of miscarriage after chorionic biopsy in the series of clinical cases mentioned above. First-trimester sampling of chorionic villi has great potential for prenatal diagnosis. Termination of pregnancy, if required, can be performed quickly and safely by vacuum aspiration.

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Received September 1, 1983

DIRECT VISION SAMPLING OF CHORIONIC VILLI DURING EXTRA-AMNIOTIC
INSTILLATION OF PHYSIOLOGIC SALINE SOLUTION
Effect on Intra-uterine Pressure and Fetal Heart Activity

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ABSTRACT

A direct vision technique has been developed for sampling chorionic villi without contamination with maternal tissue. The technique involves instilling physiologic saline solution into the extra-amniotic space, thus giving a clear view of the amniotic sac and the intended biopsy site. The effect of such instillation on intra-uterine pressure was studied in 8 women who were scheduled to undergo elective abortion by vacuum aspiration. Instillation of 100 to 150 ml of saline increased the mean intra-uterine pressure from 9 to 19 mmHg, that is, in the same range as that of the physiologic Braxton Hicks contractions. The effect on fetal heart activity when sampling chorionic villi with this technique was studied in 36 women who were cases for prenatal diagnosis. Mean fetal heart activity (beats per 15 seconds) was 36.0 before sampling, 33.2 during sampling, and 36.8 soon after the procedure had been completed. Sampling of chorionic villi under saline instillation seems thus to have only a moderate effect on intra-uterine pressure and fetal heart activity.

INTRODUCTION

The technique most commonly used for sampling chorionic villi in the first trimester is ultrasound-guided transcervical aspiration.^{1,2} One disadvantage with this technique is that the specimen obtained may be contaminated with maternal tissue. We have found it possible to obtain chorionic villi without such contamination by sampling under direct vision during continuous saline infusion.³ We report here our results of a study on the effect of such instillation on intra-uterine pressure and fetal heart activity.

MATERIAL AND METHODS

Clinical material

The clinical material included 48 women in the 9th to 13th week of gestation. Twelve of the women were to undergo elective abortion by vacuum aspiration, and the remaining 36 were cases for prenatal diagnosis. In 8 of the 12 cases for elective abortion, intra-uterine pressure was recorded during extra-amniotic instillation of 100 ml (2 cases) or 150 ml (6 cases) of saline, performed under general anesthesia; in the remaining 4, instillation of 150 ml saline was done with neither anesthesia nor analgesia and the women were asked whether they experienced any discomfort. In the 36 cases for diagnosis, fetal heart activity was registered during the sampling procedure.

The Ethics Committee of Lund University Hospital had approved the studies and informed consent was obtained from the patients.

Intra-uterine pressure study

A double catheter system was used, i.e., a No. 12 Foley catheter within which a 2 mm diameter micro-transducer catheter (Gaeltec) had been placed. In the external orifice of the Foley catheter a specially constructed occlusive plastic plug was inserted, encircling the micro-transducer catheter.

The catheters were introduced, with the Foley catheter balloon lying just inside the internal cervical orifice. The balloon was distended with 5 ml of fluid. During continuous recording of the intra-uterine pressure with a Watanabe Linear Corder, Mark III, an amount of 100 - 150 ml of saline was instilled extra-amniotically through the Foley catheter by a volumetric infusion pump (Imed, Milton Trading Estate) at a rate of 10 ml per minute.

The micro-transducer catheter was calibrated before and after the recording.

The reason for using up to 150 ml of saline in this study was our previous finding during sampling in women scheduled to undergo vacuum aspiration that the amount of saline needed to distend the extra-amniotic space, as calculated from ultrasound scans, was 150 ml at most.

Registration of fetal heart activity

Before, during, and a few minutes after sampling, fetal heart activity per 15 seconds was counted on real-time ultrasound scans made with a Hitachi EUB-25M Linear Array or Diasonics DS 20 Sector Scanner.

Before commencing this study, we had registered fetal heart activity during sampling in 48 women prior to vacuum aspiration. The result of that study did not differ significantly from those of the diagnostic cases presented here.

Chorionic villi sampling

The equipment used was a 1.7 mm diameter endoscope (Olympus Selfoscope or Dyonics Needlescope) and a specially manufactured endoscope cannula 3.0 x 4.7 mm in outer diameter (Richards Scandinavia, Mölndal, Sweden) with two attachments, one for the instillation of body-temperature saline solution and the other for the biopsy forceps (Pencil-Type Grip, Olympus or Dyonics), and a rubber hood covering the forceps attachment.

With the endoscope positioned in the cannula and the saline "drip" functioning, the set of instruments was introduced into the extra-amniotic space and directed to the base of the amniotic sac. The biopsy forceps were inserted and a piece of decidua removed. The forceps were withdrawn and, under endoscopic vision, the instrument was inserted 1 to 2 cm into the placenta. A second pair of forceps was passed through, with the jaws just emerging from the internal orifice of the cannula (the second pair was used to avoid any contamination with maternal tissue adhering to the jaws of the forceps first used). An assistant made sure with ultrasound that the tip of the instrument was located half-way between the base of the amniotic sac and the base of the placenta. The forceps were rotated once or twice, thereby entwining villi around the jaws. The jaws were then closed and, to avoid any stripping of the specimen, the forceps were held in position by an assistant while the saline "drip" was stopped, the endoscope withdrawn and the rubber hood loosened from its attachment. Finally, the forceps were carefully withdrawn.

In the first 2 women studied, the sampling procedure was done under general anesthesia; thereafter, in 6 under local anesthesia; in 14 after 15 mg Diazepam by rectal instillation 20 minutes before the procedure

and 10 mg Diazepam intravenously on commencing; and, finally, in 14 with neither anesthesia nor analgesia.

RESULTS

Intra-uterine pressure increased continuously and smoothly during the extra-amniotic saline instillation (Table I). Fig. 1 shows an illustrative case. Even during distension by 150 ml of saline, the highest pressure recorded was only 23 mmHg.

Of the 4 women who underwent saline instillation without anesthesia or analgesia, only one experienced a slight pelvic sensation when 150 ml of saline had been instilled; the other 3 women felt no discomfort.

Fetal heart activity decreased slightly during sampling but returned to pre-sampling values soon after the procedure had been completed and the saline allowed to escape (Table II).

COMMENT

Although, with this technique, the extra-amniotic space was distended with saline and the instrument inserted into the placenta during continuous saline infusion, fetal heart activity decreased only slightly. And when sampling was completed and the saline allowed to escape, the activity soon returned to pre-sampling values. It is therefore unlikely that the use of this technique has any detrimental effect on the fetus.

Even at distension by 150 ml of saline, intra-uterine pressure never exceeded 23 mmHg, thus not more than that previously recorded during the physiologic and ordinarily painless contractions of Braxton Hicks.⁴ Another finding also indicating that intra-uterine pressure increased only moderately was the absence of any painful pelvic

sensation during the saline instillation among those women who were not given analgesia.

The technique has so far been used for diagnosis in 41 cases. Of the 36 women who continued their pregnancy after sampling, 2 aborted spontaneously 3 and 5 weeks, respectively, after the sampling procedure; 4 have given birth to healthy babies; and the remaining 30 pregnancies have so far continued uneventfully. The sampling is now performed without anesthesia or analgesia. It causes little or no discomfort and is completed within a few minutes. No cervical dilatation is necessary - not even in nulliparae. The woman may return home shortly afterwards.

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Table I. Intra-uterine pressure (mmHg) during instillation of physiologic saline solution into the extra-amniotic space of 8 women scheduled to undergo vacuum aspiration in the 9th to 13th week of gestation.

Case No.*	Gestational age (weeks)	Volume of saline instilled (ml)			
		0	50	100	150
1	10	8	14	20	nd
2	9	10	10	20	nd
3	11	9	9	16	18
4	13	8	8	15	17
5	9	8	8	13	23
6	9	8	8	12	16
7	9	8	8	16	20
8	9	12	12	16	18
Mean		9	10	16	19

*Cases numbered in order in which they were studied.

nd = not done

Table II. Fetal heart activity (beats per 15 seconds) during chorionic villi sampling in 36 cases for prenatal diagnosis.

	Fetal heart activity		
	range	mean	SD
Before sampling	30 - 40	36.0	2.1
During sampling	25 - 37	33.2	2.6
A few minutes after sampling	32 - 41	36.8	2.2

LEGEND

Fig.1. Intra-uterine pressure recording and ultrasound scanning during instillation of 150 ml of physiologic saline solution into the extra-amniotic space of a nine-week uterus (case No. 7 in Table I). Note the slight and smooth increase in intra-uterine pressure and the absence of any change in amniotic sac outline throughout instillation.

